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(21) International Application Number: PCT/SE98/02036 (22) International Filing Date: 11 November 1998 (11.11.98) (30) Priority Data: 9704141-2 12 November 1997 (12.11.97) SE (71) Applicant (for all designated States except US): SBL VACCIN AB [SE/SE]; Lundagatan 2, S-105 21 Stockholm (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): FRYKBERG, Lars [SE/SE]; Stabby Alle 7 C, S-752 29 Uppsala (SE). JACOBSSON, Karin [SE/SE]; Stabby Alle 9 C, S-752 29 Uppsala (SE). (74) Agents: NILSSON, Brita et al.; AB Stockholms Patentbyrå AB, Zacco & Bruhn (publ), P.O. Box 23101, S-104 35 Stockholm (SE).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>	
(54) Title: IgG-BINDING PROTEIN FROM <i>STAPHYLOCOCCUS</i> AND NUCLEOTIDE SEQUENCE ENCODING THIS PROTEIN (57) Abstract A recombinant DNA molecule coding for a protein expressed by a <i>Staphylococcus aureus</i> bacterium, comprising the nucleotide sequence SEQ ID NO: 1 or a homologous sequence, or a partial or homologous sequence of SEQ ID NO: 1 coding for a polypeptide fragment comprising at least 15 amino acid residues, is described. Further, a protein expressed by such a bacterium or a polypeptide fragment comprising at least 15 amino acid residues, comprising the amino acid sequence SEQ ID NO: 2 binds IgG and apolipoprotein H. Examples of the polypeptide fragments comprise the SEQ ID NO: 3 through 6. These proteins and polypeptide fragments may be coupled to an inert carrier or matrix. Vectors comprising such a DNA molecule or the corresponding RNA molecule, and antibodies specifically binding to a polypeptide having an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6, are also disclosed. The DNA or RNA molecules, the vectors and the antibodies mentioned may all be used in different types of vaccines against Staphylococcal infections. Moreover, a method of isolating and/or purifying apolipoprotein H from a liquid medium, especially from serum, is described.		

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IgG-BINDING PROTEIN FROM *STAPHYLOCOCCUS* AND NUCLEOTIDE SEQUENCE ENCODING THIS PROTEIN

The present invention relates to a new protein and a nucleotide sequence encoding said protein. More precisely, the invention relates to a DNA molecule coding for a protein expressed
5 by a bacterium of the genus *Staphylococcus aureus*, said protein and polypeptide fragments of said protein. Vectors comprising the nucleotide sequence coding for the protein, the protein and fragments thereof, and antibodies specifically binding to the protein may all be used for different vaccines against Staphylococcal infections in mammals. The invention also relates to a method of isolating and/or purifying apolipoprotein H from e.g serum with an immobilised protein or
10 polypeptide of the invention.

Background of the invention

Staphylococcus aureus is a pathogen responsible for a wide variety of diseases in humans and animals, including endocarditis, osteomyelitis, wound sepsis and mastitis. The bacterium produces several potential virulence factors such as alpha-, beta-, gamma- and delta-toxins, toxic
15 shock syndrome toxin (TSST), enterotoxins, leucocidin, proteases, coagulase and clumping factor.

It is generally accepted that adhesion to tissues is required for bacterial colonisation to occur. For this purpose staphylococci express surface adhesins, which interact with host matrix proteins such as fibronectin, vitronectin, collagen, laminin and bone sialoprotein. In addition,
20 staphylococci are able to bind several serum proteins, such as IgG, fibronectin, fibrinogen, and thrombospondin, possibly masking the bacteria from the immune system of the host. However, the contribution and importance of each of these binding functions in different infections is still unclear.

The most studied receptor in *S. aureus* is protein A, a cell wall-associated protein, which
25 binds to the Fc- and the Fab-regions of IgG from several species. Protein A in strain 8325-4 consists of five consecutive, highly homologous domains, all with IgG-binding activity, followed by a region anchoring the protein in the cell wall (Uhlén *et al*, 1984). IgG-binding ability is common among clinical strains of *S. aureus* suggesting an important function in pathogenesis. It has been assumed that the IgG-binding capacity is mediated by protein A only.

30 However, the present inventors recently identified a nucleotide sequence in *S. aureus* strain 8325-4 encoding a polypeptide, clearly distinguishable from protein A, which binds IgG in a non-immune fashion (Jacobsson & Frykberg, 1995). An IgG-binding protein fragment having an amino acid sequence of 84 aa was disclosed. However, the amino acid sequence of the full

length protein and the properties other than the IgG-binding ability of the 84 aa fragment were not known or even suggested. No nucleotide sequence coding for said protein has been disclosed or suggested prior to the present invention.

5 Diseases caused by Staphylococcal infections are often treated with antibiotics. As is well known in the art, these microorganisms can develop antibiotic resistance. Therefore, the use of vaccines to prevent or contain the spread of infection would be desirable. At present, there is no vaccine on the market that gives full protection. The present invention provides new immunologically active components for the production of vaccines against Staphylococcal infections.

10 Description of the invention

The present invention is based on cloning and nucleotide sequence determination of a complete gene (*sbi*) encoding a novel IgG-binding protein. The gene encodes a protein of 436 amino acids, denoted protein Sbi, with one IgG-binding domain that exhibits an immunoglobulin-binding specificity similar to protein A and without the typical Gram-positive cell wall anchoring sequence LPXTG (Schneewind *et al*, 1995) suggesting that the protein is not anchored in the cell wall. Analysis of other *S. aureus* strains shows that this gene is not unique for strain 8325-4. For instance, the Sbi-protein is highly expressed in strain Newman 4, which shows that the IgG-binding activity observed in *S. aureus* is not mediated only by protein A. In fact, this (*sbi*) gene is present in all tested strains of *S. aureus*.

20 Further, it has now been revealed that the Sbi protein of the invention binds apolipoprotein H, a major serum component, in addition to IgG. Hitherto, no bacterial protein binding to apolipoprotein H has been reported. Therefore, neither is this combination of the protein binding to these two serum components previously known. The portion of the protein which binds to IgG is located near the N-terminal of the protein, whereas the middle portion binds to apolipoprotein H. This enables the use of the protein, or an appropriate polypeptide fragment, in immobilised form for the isolation and/or purification of apolipoprotein H.

25 Thus, one aspect of the present invention is directed to a recombinant DNA molecule coding for a protein expressed by a bacterium of the genus *Staphylococcus aureus*, comprising the nucleotide sequence SEQ ID NO:1, defined in the sequence listing and the claims, or a homologous sequence to SEQ ID NO:1 coding for said protein, or a partial or homologous sequence of the sequence SEQ ID NO:1 coding for a polypeptide fragment of said protein comprising at least 15 amino acid residues.

This recombinant DNA molecule may be inserted into plasmids, phages or phagemides for the expression/production of the protein or protein fragments.

Another aspect of the invention is directed to a protein expressed by a bacterium of the genus *Staphylococcus aureus* or a polypeptide fragment of said protein comprising at least 15 amino acid residues other than the 84 aa fragment at the position 38 - 121, which protein comprises the amino acid sequence SEQ ID NO: 2, defined in the sequence listing and the claims, or a homologous sequence to the sequence SEQ ID NO: 2 comprising a few mismatches in the amino acid sequence of SEQ ID NO: 2, or polypeptide fragments of said homologous sequence comprising at least 15 amino acid residues.

The disclaimer of the 84 aa fragment at the position 38 - 121 of the SEQ ID NO: 2 is made because, as already mentioned, it has been previously disclosed (Jacobsson & Frykberg, 1995).

It is well known in the art that there may be a few mismatches of amino acids residues in the amino acid sequence of a protein while the protein still retains its major characteristics.

The mismatches may be replacements of one or several amino acids, deletions of amino acid residues or truncations of the protein. Such mismatches occur frequently in genetic variations of native proteins. It is believed that up to 15% of the amino acid residues may be replaced in a protein while the protein still retains its major characteristics. The protein of the invention comprises 436 amino acid residues, and therefore up to 66 mismatches would be acceptable.

However, preferably there will be less than 20, more preferably less than 10, and most preferably less than 5 mismatches in the amino acid sequence of the protein of the invention.

The polypeptide fragments of the protein of the invention should comprise at least 15 amino acid residues to be sure that the fragments are not found in other known proteins.

These fragments may be used e.g. as probes, diagnostic antigens, and vaccine components, possibly coupled to carriers.

In an embodiment of this aspect of the invention a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 3, defined in the sequence listing and the claims. This polypeptide fragment lacks the signal sequence of the SEQ ID NO:1.

In another embodiment a polypeptide fragment of the protein according to the invention has an amino acid sequence SEQ ID NO: 4, defined in the sequence listing and the claims. This polypeptide fragment binds apolipoprotein H.

In yet another embodiment a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 5, defined in the sequence listing and the claims. This 120 aa polypeptide fragment binds IgG. It was chosen for immunisation purposes, instead of the known IgG binding 84 aa fragment, since once the whole amino acid sequence was deduced, it became evident there were sequence similarities suggesting two IgG binding domains.

In still another embodiment a polypeptide fragment of the protein according to the invention has the amino acid sequence SEQ ID NO: 6. This polypeptide fragment binds apolipoprotein H, and has been used for isolation and purification of said serum protein.

10 In a preferred embodiment of this aspect of the invention the protein or polypeptide according to the invention is coupled to an inert carrier or matrix. The carrier may be e.g. plastic surfaces, such as microplates, beads etc.; organic molecules such as biotin; proteins, such as bovine serum albumin; peptide linkers, polypeptides e.g. resulting in fusion proteins. The matrix may be particles used for chromatographic purposes, 15 such as Sepharose®.

A further aspect of the invention is directed to a vector selected from the group consisting of plasmids, phages or phagemides comprising a nucleotide sequence according to the invention.

20 These vectors may be used for the production of the proteins or polypeptides of the invention. They may also be used in vaccines.

Yet another aspect of the invention is directed to antibodies specifically binding to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:6. The specific binding of binding of an antibody to an amino acid sequence of the invention requires e.g. an affinity constant of at least 10^7 liters/mole, 25 preferably at least 10^9 liters/mole.

The antibodies of the invention may be monoclonal or polyclonal. They may be used in diagnostic tests, but preferably in vaccines for passive immunization.

30 Still another aspect of the invention is directed to the use of a protein or polypeptide according to the invention, optionally in immobilised form, as an immunising component in the production of a vaccine against Staphylococcus infections.

Another use aspect of the invention is directed to the use of a vector according to the invention for the production of a vaccine against Staphylococcal infections.

Yet another use aspect of the invention is directed to the use of antibodies according to the invention for the production of a vaccine for the passive immunisation of a mammal against *Staphylococcus* infections.

5 An additional aspect of the invention is directed to a vaccine against *Staphylococcal* infections comprising as an immunising component a protein or polypeptide according to the invention, optionally in immobilised form.

Another vaccine aspect of the invention is directed to a vaccine against *Staphylococcal* infections comprising a vector according to the invention.

10 A DNA molecule, or the corresponding RNA derived from the present sequence, as described in claim 1, may be used in a vector for vaccine purposes. Examples of suitable forms of administration include intravenous, percutaneous, and intramuscular administration.

Yet another vaccine aspect of the invention is directed to a vaccine for the passive immunisation of a mammal, especially a human being, against *Staphylococcus* infections comprising antibodies according to the invention.

15 One embodiment of the invention comprises the passive immunization of patients with an impaired immune defence or patient awaiting major surgery, such as patients in line for an organ transplantation or awaiting the insertion of a prosthetic device, such as a hip prosthesis or similar major surgical intervention. According to the present invention, a high dose of antibodies against the novel protein can be administered to any patient before or at the
20 time of hospitalisation, in order to prevent *Staphylococcus* infection.

The vaccines may contain other ingredients selected with regard to the intended administration route, and these ingredients are chosen by the vaccine manufacturer in collaboration with pharmacologists. Examples of administration routes include intravenous administration, percutaneous administration, oral and nasal administration.

25 A further aspect of the invention is directed to a method of prophylactic and/or therapeutic treatment of *Staphylococcus* infections in a mammal comprising administration to said mammal of an immunologically effective amount of a vaccine according to any one of the vaccines of the invention.

30 Still another aspect of the invention is directed to a method of isolating and/or purifying apolipoprotein H from a liquid medium, especially from serum, comprising chromatographic separation of apolipoprotein H from said liquid medium with an immobilised protein or polypeptide according to the invention as stationary phase.

In a preferred embodiment of the invention column chromatography is used for the isolation/purification of apolipoprotein H from blood serum. The protein or polypeptide of the invention is coupled to e.g. Sepharose® and is used as packing material for the column. The apolipoprotein H-containing serum is brought into contact with the immobilized protein or polypeptide and the apolipoprotein H is adsorbed. Finally, the apolipoprotein H is eluted from the column.

Description of the drawings

Figure 1 shows a) the cloned 3 kb *Pst*I - *Xba*I fragment from *S. aureus* strain 8325-4 containing the *sbi*-gene of the invention (clone pPX1), and b) schematic drawing of the protein Sbi aligned with the peptides encoded by the phagemid clones isolated by panning against IgG. Stars indicate that the clone was isolated several times independently. Ig4 represents the clone isolated earlier (Jacobsson & Frykberg, 1995). S denotes the signal sequence and the shaded bar represents a proline-rich region.

Figures 2 a + 2 b show the complete nucleotide sequence of the *sbi*-gene of the invention from *S. aureus* 8325-4, and the deduced amino acid sequence of the encoded protein. Features of the sequence are indicated as follows: underbar, putative promotor sequences; double underbar, possible ribosomal binding site; overbar, putative transcriptional stop signal (inverted repeat); single vertical arrow indicates the cleavage site of the signal sequence and in bold the repeated proline residues. Also shown in bold is the minimal IgG-binding domain as deduced by shot gun phage display mapping.

Figure 3 shows the specificity of the binding domain of the protein Sbi (clone Ig4) for various IgGs and human IgG3κ, IgG3λ, IgM and IgA. Each column represents a mean value of two independent experiments with the same phage stock. The y-axis shows the number of c.f.u. in 50 µl of the eluate.

Experiments

Analysis of IgG binding activity in *S. aureus* by Western blots usually reveals more than one protein band, which interact with IgG, and it has been assumed that these polypeptides represent breakdown products of protein A. In view of the present results, it is not surprising that protein Sbi has escaped detection. For example, analysis of the expression of the protein Sbi in *S. aureus* has been hampered by the ability of protein A to interact with IgGs from most mammalian species. Furthermore, both proteins migrate similarly in SDS-PAGE and have the same specificity for all tested immunoglobulins. As disclosed in Example 4 below, the

commercially available anti-protein A antibodies cross-react with full-length protein Sbi but not with MAL-Sbi Δ (SEQ ID NO:4) (lacking the known IgG-binding domain). Most likely, the cross-reaction is not unique to this source of antibodies and may have contributed to the failure to identify protein Sbi. Now the antibodies directed against the protein Sbi of the invention
5 produced in chicken allows discrimination between expression of protein A and protein Sbi.

The present inventors have cloned and sequenced the *sbi*-gene from *S. aureus* strain 8325-4. The detection of protein Sbi, expressed from its own promoter in *S. epidermidis* and as a MAL-fusion in *E. coli* with HRP-labelled IgG, proves that this gene encodes a second IgG-binding protein. This protein consists of 436 amino acids (SEQ ID NO:2) and contains a signal
10 sequence but lacks the cell wall sorting LPXTG motif, suggesting that the protein is not anchored in the cell-wall. However, in strain Newman 4, which produces high amounts of protein Sbi, no protein Sbi is detected in the culture growth medium (data not shown). Instead, protein Sbi is released from the cell surface by addition of sample buffer (Example 4), in contrast, release of protein A requires treatment with lysostaphin (Example 4). This indicates that protein Sbi is
15 associated to the cell surface by a different mechanism. There are also other examples of cell surface associated proteins that lack the LPXTG motif, such as the *S. aureus* elastin-binding protein (Park et al 1996) and MHC class II analogue (Jönsson et al 1995).

Mapping of protein Sbi by shot-gun phage display strongly suggests that the protein has only one IgG-binding domain with a deduced minimal binding domain of 52 amino acids.

20 Furthermore, expression and analysis of NH₂-terminally truncated Sbi-protein, i.e the C-terminal part of the protein, consisting of aa 143-436 (SEQ ID NO:4), shows that no IgG-binding activity is located in this part of the protein (Example 4). The IgG-binding domain shows a significant homology to the IgG-binding repeats of protein A (Fig. 3).

A phage-stock made from the originally isolated clone, Ig4 (aa 38-121) (Fig. 1b), was
25 used in an analysis of the immunoglobulin species reactivity (Fig. 3). A comparison between data on the specificity of protein A (Boyle, 1990) and the results for clone Ig4 (Fig. 3) shows, that the two proteins exhibit a very similar Ig-binding profile. In addition, a study using one or two binding domains from protein A (clones Ig7 (domain C) and Ig1 (D-A) described by Jacobsson & Frykberg (1995)) gave an Ig-binding profile similar to that of Ig4 (data not shown).
30 The display of protein domain(s) on the phage surface offers a quick and sensitive method for analysing specific binding to other molecules.

Further, the present protein has been shown to bind to another serum protein, apolipoprotein H, also known as β 2-glycoprotein I. Hitherto, no bacterial protein binding to

apolipoprotein H has been reported. This finding is unexpected and opens new possibilities to use the protein for isolation/purification of apolipoprotein H for research laboratories and for the production of antibodies against the apolipoprotein H binding portion of the protein. These antibodies may be used as components in vaccines for passive immunisation of mammals against

5 *Staphylococcus* infections.

Examples

Bacterial strains, growth conditions, vectors and helper phage

The bacterial strains used are listed in Table 1. Phage R408 (Promega) was used as the helper phage for production of phage stocks. *E. coli* containing the pUC18 or pMAL-c2 vectors
10 (New England Biolabs) were selected on LA-plates (Luria-Bertani (LB)-broth with 1.5% agar and 50 µg ampicillin ml⁻¹) and grown in LB-broth supplied with 50 µg ampicillin ml⁻¹. *E. coli* containing the phagemid vector pHEN1 (Hoogenboom *et al*, 1991), were grown in the same medium supplemented with 1% glucose (w/v). Staphylococcal strains were grown in Tryptone Soya Broth (TSB) (Oxoid). *S. epidermidis* containing pBR473 was grown in the same medium
15 containing 20 µg ml⁻¹ of chloramphenicol.

Table 1. Bacterial strains

20	Species	Strain	Characteristics and use
	<i>E. coli</i>	TG1	F ⁺ and amber suppressing. Used for construction of the phage library and production of phage stocks.
25		MC1061	Used for all other DNA manipulations.
	<i>S. aureus</i>	8325-4	NCTC 8325 cured from prophages
30		Wood 46	Protein A-negative reference strain
		Newman 4	Spontaneous mutant of strain Newman with enhanced production of fibronectin-binding protein
35		Cowan I	NCTC 8350, high level producer of cell-wall- bound protein A.
	<i>S. epidermidis</i>	247	

40

Example 1: Cloning and sequencing of the *sbi* gene encoding an IgG-binding protein

Restriction and modification enzymes were purchased from Promega, Amersham International, or Boehringer Mannheim. Oligonucleotides were synthesized by Scandinavian
 5 Gene Synthesis AB or Pharmacia Biotech and are listed in Table 2.

Table 2. Oligonucleotides.

10	Name	Use	Sequence
	Pe	Sequencing of pHEN1 clones	5'- TTG CCT ACG GCA GCC GCT GAA -3'
	My	Sequencing of pHEN1 clones	5'- TGC GGC CCC ATT CAG ATC CTC -3'
	Olg1	Sequencing of <i>sbi</i>	5'- CTC CAT ATA GTA CTT CCT TA-3'
	Olg2	Sequencing of <i>sbi</i>	5'- GAG ATT GCA TCA TTT GCT GA-3'
15	Olg3	Sequencing of <i>sbi</i>	5'- GTA ACC ATA GTT AAA TGA AT-3'
	Olg4	Sequencing of <i>sbi</i>	5'- CGA TAA ATC AGC AGC ATA TG-3'
	Olg5	Sequencing of <i>sbi</i>	5'- CAA TCA CCA CAA ATT GAA AA-3'
	Olg6	Sequencing of <i>sbi</i>	5'-TGG TGC TTG TAG TGG AAA AG-3'
	Olg8	PCR for MAL-E fusions	5'-AGT GGA TCC ACG CAA CAA ACT TCA ACT AAG CA-3'
20	Olg9	PCR for MAL-E fusions and constr. of <i>sbi</i> -probe	5'- AAT GTC GAC AAA CTA GAG AAG ATA TTT TTG A-3'
	Olg10	PCR for MAL-E fusions and constr. of <i>sbi</i> -probe	5'- TAG GAT CCG TAC AAT CTT CTA AAG CTA AAG A-3'

25 All DNA manipulations were performed using standard methods (Sambrook *et al*, 1989), except ligations and small scale plasmid preparations, for which the Ready to Go-ligation kit (Pharmacia Biotech) and WizardTM Miniprep DNA Purification systems (Promega), respectively, were used according to the manufacturers' instructions. Plasmids were introduced into *E. coli* and staphylococci by electrotransformation. Staphylococcal chromosomal DNA was
 30 prepared according to Lindberg *et al* (1972).

DNA was sequenced according to the dideoxy chain termination method using the Sequenase^R version 2.0 DNA sequencing kit from United States Biochemical. Restriction sites

shown in Fig. 1 were used for construction of subclones used in determination of the nucleotide sequence. One additional clone, pHSBB7, was made (not shown) by *Bal31* exonuclease digestion from the unique *HindIII* site in the 3' direction of the gene, and sequenced. Different oligonucleotides were used as primers for determining the sequence of both DNA strands (Table 5 2). The PC-gene program (Intelligenetics) was used for the handling of the sequences. The EMBL, GeneBank, SWISS-protein and PIR databases were searched for sequence-homologies:-

To express the Sbi protein, two constructs were made in the pMAL-c2-vector. Primers Olg8 and 9 were used to PCR-amplify the DNA encoding full-length protein lacking the signal sequence (aa 33-436, SEQ ID NO: 3). Primers Olg9 and 10 were used to PCR-amplify the DNA 10 encoding a truncated version (aa 143-436, SEQ ID NO: 4) lacking also the known IgG-binding domain. The obtained PCR-products were digested with the restriction enzymes *BamHI* and *Sall* and ligated into the vector cleaved with the same enzymes and transformed into *E. coli*.

Southern blots and hybridizations were performed according to Sambrook *et al* (1989), using a *NcoI-XhoI* fragment, i.e. the complete insert from clone Ig4, (Jacobsson & Frykberg, 15 1995) ³²P-labelled by random priming for detection and cloning of the *sbi*-gene. For detection of the *sbi*-gene in different *S. aureus* strains, the PCR-fragment obtained by using Olg9 and 10 (see above) was ³²P-labelled by random-priming. The hybridisation was carried out at 50°C and the washing at 65°C in 0.1 x SSC (20xSSC= 3.0M NaCl and 0.3M Na-citrate) and 0.1 % SDS.

The original clone (Fig. 1b, clone Ig4) expressing an IgG-binding polypeptide was earlier 20 isolated from a shot-gun phage display library made from *S. aureus* strain 8325-4 (Jacobsson & Frykberg, 1995). The insert from this clone was used as a probe for identification and subsequently for cloning of the complete gene from *S. aureus* strain 8325-4. Chromosomal DNA was digested with *PstI* and *XbaI*, and the DNA fragments were separated by agarose gel electrophoresis followed by blotting onto a nitrocellulose filter. Hybridization with the *NcoI-XhoI* 25 fragment, derived from Ig4, showed that the gene of the invention resided on a fragment of approximately 3 kb in size. DNA fragments of this size were purified by agarose gel electrophoresis and cloned into the pUC18 vector. One clone, pPX1, hybridizing with the probe was further characterized by restriction enzyme analysis. Fig. 1 schematically shows the 3 kb *PstI-XbaI* DNA fragment containing the *sbi*-gene and the different restriction enzymes used for 30 subcloning.

Fig. 2 shows 1620 nt of the 3 kb *PstI-XbaI* DNA fragment. The coding sequence starts with a Met at nucleotide position 181 and ends with a stop codon at position 1488 (SEQ ID NO:1), encoding a protein of 436 amino acids (SEQ ID NO: 2), including a typical signal

peptide with a putative cleavage site after amino acid 29. The gene has the normal features associated with a functional gene, putative promotor sequences, a possible ribosomal binding site and an inverted repeat located after the translation termination stop codon. A proline rich sequence, containing eight prolines repeated every fifth amino acid, starts at position 267. Such sequences are normally found within cell wall spanning domains. However, in this case the proline rich region is not followed by the cell wall sorting LPXTG motif (Schneewind *et al*, 1995).

Example 2: Mapping of the IgG-binding domain in protein Sbi

The library was constructed from the cloned *sbi*-gene essentially as described (Jacobsson & Frykberg, 1995). In short, the DNA from clone pPX1 (Fig. 1a) was sonicated and DNA fragments in size of approximately 50 - 300 bp were isolated by preparative gel electrophoresis. The fragments were made blunt-ended with T4 DNA polymerase and ligated into the phagemid pHEN1, previously digested with *Pst*I, made blunt-ended, and dephosphorylated with calf intestine alkaline phosphatase. The ligation was made using 1 µg of vector and 1 µg of DNA fragments and the mixture was transformed into *E. coli* TG1. The transformants were grown over night in LB supplied with 50 µg ampicillin ml⁻¹ and 1% glucose (w/v) and thereafter infected with helper phage R408 at a MOI of 20. After 1 hour the culture was diluted and ampicillin added to a final concentration of 50 µg ml⁻¹. After 5 hours of growth at 37°C, the bacteria were pelleted and the supernatant, containing the phages, was sterile filtered.

The library was affinity selected against human IgG (Kabivitrum) and positive clones were identified using labelled IgG as described (Jacobsson & Frykberg, 1995).

In order to determine the exact position of the IgG-binding domain in protein Sbi a shot-gun phage display library was made from clone pPX1. After panning the library against immobilized human IgG, *E. coli* TG1 cells were infected with the eluted phage and the bacteria were spread on LA-plates containing ampicillin. Amp^R colonies were analysed for binding of HRP-labelled human IgG. Positive clones were isolated and the nucleotide sequences of the different inserts were determined. As seen in Fig. 1 all clones had inserts derived from the same part of *sbi*, suggesting that the encoded protein has only one IgG-binding domain. From the sequences of the binding clones in Fig. 1, the minimal IgG-binding domain is deduced to consist of 52 amino acids.

Example 3: Analysis of the immunoglobulin specificity of protein Sbi

To determine the specificity of protein Sbi, a phage-stock was prepared as described above from clone Ig4, encoding the IgG-binding domain of protein Sbi (Jacobsson & Frykberg,

1995). The stock was diluted to 10^8 phagemid particles ml^{-1} and 100 μl was panned as described (Jacobsson & Frykberg, 1995) against human IgG, IgM, IgA, IgG3 κ , IgG3 λ as well as IgG from rat, goat, pig, cow, sheep, horse, guinea pig, dog, rabbit and chicken (Sigma), immobilized in microwells at a concentration of $50 \mu\text{g ml}^{-1}$. BSA was included as a negative control. The number of bound phagemid particles was determined as c.f.u. after infection of *E. coli* TG1 cells with the phage eluted at pH 2.

To analyse the Ig-binding properties of protein Sbi, clone Ig4 encoding the IgG-binding domain, was used to produce phage-particles displaying the binding domain on the surface. These phages were panned against different immunoglobulins and the number of binding phagemid-particles was determined and used as a measure of the binding ability (Fig. 3).

Example 4: Protein purification and electrophoresis

The MAL-fusion proteins, MAL-Sbi (aa 33- 436, SEQ ID NO:3) and MAL-Sbi Δ (aa 143-436, SEQ ID NO:4), were purified from *E. coli* lysates on an amylose-resin according to the manufacturer's instructions (New England Biolabs). MAL-Sbi was further purified on IgG-Sepharose[®] according to the manufacturer's instructions (Pharmacia Biotech).

Antibodies against protein Sbi, developed in chickens, were obtained through Immunsystem AB (Uppsala, Sweden) (four immunizations of 50 μg MAL-Sbi Δ -protein). The antibodies were affinity-purified on immobilized MAL-Sbi Δ -protein and labelled with Horse-radish peroxidase (HRP) (Boehringer Mannheim). Commercially available HRP-labelled chicken antibodies against protein A were ordered from Immunsystem AB.

To further characterize the protein encoded by the *sbi*-gene the *Pst*I-*Xba*I fragment from pPX1, encoding the complete protein, was made blunt ended and inserted into the *Sma*I site in the shuttle plasmid pBR473. The construct, pShPX1, was first introduced into the restriction negative *S. aureus* strain 113 and plasmid DNA was prepared from one chloramphenicol resistant colony. The plasmid was then transferred to *S. epidermidis* strain 247, known to be negative in IgG-binding as all coagulase negative staphylococci, and chloramphenicol resistant colonies were screened for binding of HRP-labeled IgG. All clones analysed had received IgG-binding capacity and one of the clones, SePX1, was chosen for further studies. Chromosomal DNA was prepared from this clone, cleaved with *Sna*BI and *Hind*III and analysed by Southern blot hybridization, which showed that the clone contained the *sbi* gene as expected (data not shown).

The bacterial cells in over night-cultures of Newman 4 (2 ml), Cowan I (2 ml) and *S. epidermidis* containing pShPX1 (40 ml) were collected by centrifugation and washed once in

PBS (phosphate buffered saline pH 7.4). Newman 4- and *S. epidermidis*-cells were resuspended in 40 µl PBS followed by addition of 40 µl 2x sample-buffer (1x buffer = 125 mM Tris-HCl pH 6.8, 10% glycerol, 5% (v/v) β-mercaptoethanol, 2 % SDS and 0.1% bromophenol blue) and the samples boiled for 2 minutes. To release protein A, the same amount of cells in PBS was treated with lysostaphin (0.1 mg/ml, Sigma) for 5 minutes at 37°C before addition of the sample-buffer and boiling for 2 minutes. The 1µl of the samples were analyzed by SDS-PAGE using the Phast-system (Pharmacia Biotech). Also included were 1/40 µg protein A (Pharmacia Biotech), 1/20 µg MAL-Sbi (SEQ ID NO:3) and 1/20 µg MAL-SbiΔ (SEQ ID NO:4). The proteins were blotted onto nitrocellulose-filters (Schleicher&Schuell) and proteins detected using HRP-labelled affinity purified rabbit anti-chicken (diluted 1/500, Sigma), anti-Sbi (10 µg/ml) or anti-protein A (15 µg/ml). Bound antibodies were detected using 4-chloro-1-naphtol (Serva).

To further characterize the protein encoded by the *sbi*-gene, two clones were made in the pMAL-c2 vector, expressing the mature full length protein (MAL-Sbi, aa 33 - 436, SEQ ID NO:3) and a truncated protein (MAL-SbiΔ, aa 143 - 436, SEQ ID NO:4) lacking the known IgG-binding domain. The purified products were analysed by SDS-PAGE and Western blot together with commercially available protein A, cell surface extract of *S. epidermidis* containing pShX1 and *S. aureus* Newman 4, as well as lysed Newman 4 and Cowan I cells. Three duplicate gels were blotted onto nitrocellulose-filters and the blots developed with HRP-labelled rabbit IgG for detection of protein A and Sbi, anti-protein A and anti-Sbi, respectively.

Western blot analysis of purified proteins shows that antibodies against MAL-SbiΔ (SEQ ID NO:4) recognise both MAL-Sbi (SEQ ID NO:3) and MAL-SbiΔ (SEQ ID NO:4) but not protein A. Commercially available antibodies against protein A (Immunsystem AB, Uppsala) recognise both protein A and MAL-Sbi but not MAL-SbiΔ. This means that the antibodies against protein A recognise the IgG-binding domain in protein Sbi. The antibodies against the polypeptide fragment MAL-SbiΔ of the protein Sbi are specific for the protein Sbi. Western blot analysis of lysates of two different *S. aureus* strains show that the antibodies against MAL-SbiΔ (SEQ ID NO:4) only recognise protein Sbi and no other staphylococcal protein.

Example 5: Occurrence of the *sbi*-gene in staphylococcal strains

Chromosomal DNA was isolated from *S. aureus* strains 8325-4, Cowan I, Newman 4 and Wood 46. The DNAs were digested with the enzyme *Hind*III followed by agarose gel

electrophoresis and then blotted onto a nitrocellulose filter. The Southern blot shows that the *sbi*-gene is present in all four *S. aureus* strains tested.

Example 6: Purification of apolipoprotein H

Purified MAL-SbiΔ (SEQ ID NO:4) was immobilised on CNBr-activated Sepharose®

5 (Pharmacia Biotech) according to the manufacturer's instruction. Ten ml rat (ICN), bovine (Life Technologies) or human (Uppsala university hospital) serum was diluted 20 times in PBS and passed over the column. After extensive washing with acetate-buffer pH 5,5, the bound protein was eluted in acetate buffer pH 2.7. The eluate was lyophilised and the protein dissolved in water.

10 The proteins were analysed by SDS-PAGE and blotted onto nitrocellulose-filters (Schleisher-Schuell) together with commercially available human apolipoprotein H (ICN). The proteins were detected with rabbit anti-human apolipoprotein H antibodies (Chemicon) and HRP-labelled goat anti-rabbit antibodies (Santa Cruz Biotechnology). An identical Western blot was treated with purified MAL-Sbi after which HRP-labelled chicken antibodies
15 against protein Sbi was added. Both filters were developed with 4-chloro-1-naphtol.

These results show that in addition to IgG, protein Sbi binds apolipoprotein H from various mammals and that it can be used for purification of apolipoprotein H.

Example: 7 Purification of apolipoprotein H, and immunisation with protein fragments

Apolipoprotein H from human and bovine sera were also purified on SbiApoB (aa
20 145-267, SEQ ID NO:6). This domain is used for immunisation in different animals (rat and guinea pig) to investigate if it confers protection against *S. aureus* infections. The recombinant protein SbiIgGB (31-150, SEQ ID NO:5) is also included in this immunisation study.

The Impact™ T7- system from New England Biolabs was used for construction
25 and purification of SbiIgGB (aa 31-150, SEQ ID NO:5) and SbiApoB (aa 145-267, SEQ ID NO: 6). The corresponding parts of the *sbi*-gene was amplified by PCR using the oligonucleotides IgG1pr (5'- CAT GCC ATG GAA AAC ACG CAA CAA ACT TCA- 3'), IgG2pr (5'- TTC TTT AGC TTT AGA AGA -3'), Apo1pr (5'- CAT GCC ATG GAA TCT TCT AAA GCT AAA GAA CGT- 3') and Abobin (5'- TGG CGC CAC TTT CTT TTC
30 AGC-3'). The PCR-products were digested with *Nco*I and treated with T4 polynucleotide kinase and ligated into the pTYB4 vector. The vector had previously been digested with the *Nco*I and *Sma*I and treated with alkaline phosphatase. The constructions were transformed

into BL21(DE3)pLysS-cells and protein was then purified according to the manufacturer's instructions.

The purified SbiApoB was coupled to a Hitrap® affinity column (Amersham Pharmacia BioTech). Bovine serum (Life Technologies) was applied onto the column. After
5 extensive washing with PBS (phosphate buffered saline) supplied with 0.05% Tween20, bound protein was eluted in 0.1 M glycine pH 3.0. The purified protein was analysed by SDS-PAGE and Western-blotting and was shown to be recognised by rabbit anti-human apolipoprotein H antibodies (Chemicon).

Example: 8 Passive immunisation with protein fragments

10 As prophylaxis or for treatment of acute infections in a patient, passive immunisation may be effected by administration of antibodies directed against the protein Sbi. These antibodies can be obtained by immunisation of horses with a dose of 50-1000 µg of the polypeptide fragments SEQ ID NO: 4 and/or SEQ ID NO:6 of the protein Sbi, optionally coupled to a carrier, at three to four separate occasions, followed by purification of the total
15 antibody fraction and/or the specific antibody fraction directed against the non-IgG binding portions of the protein Sbi from serum.

In addition, antibodies can be obtained by immunisation of chickens with 10-100 µg of the polypeptide fragments SEQ ID NO: 4 and/or SEQ ID NO:6 of the protein Sbi, optionally coupled to a carrier proteins per immunisation, at 3-4 separate occasions. The
20 specific antibodies against the non-IgG binding portions of the protein Sbi can then be purified from the eggs and used for passive immunisation. Alternatively, the raw eggs can be consumed to give passive protection.

Finally, antibodies can also be isolated from humans that have recovered from a staphylococcal infection by purification of the total antibody fraction and/or the specific
25 antibody fraction directed against the non-IgG binding portions of the protein Sbi from serum.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this
30 art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

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Claims

1. Recombinant DNA molecule coding for a protein expressed by a bacterium of the genus *Staphylococcus aureus*, comprising the nucleotide sequence SEQ ID NO:1

5	AGTACTTCCT	TACTTAAAT	ACGCTGAATG	TTCTGAATTA	AACGCTTTTT	TACATAGTTA	60
	ACACTAGTTA	ATCTATTAGT	TAACATTAGT	TAATAATTAG	TTAATTTCCA	TTGTATTCT	120
	CATGTGATAA	ATTCTAAAAG	CATACAATAA	ATTTAATATG	TAAAAAGAAA	GGGAATACAC	180
	ATGAAAAATA	AATATATCTC	GAAGTTGCTA	GTTGGGGCAG	CAACAATTAC	GTTAGCTACA	240
10	ATGATTTCAA	ATGGGGAAGC	AAAAGCGAGT	GAAAACACGC	AACAAACTTC	AACTAAGCAC	300
	CAACAACACT	AAAACAACTA	CGTAACAGAT	CAACAAAAAG	CTTTTATCA	AGTATTACAT	360
	CTAAAAGGTA	TCACAGAAGA	ACAACGTAAC	CAATACATCA	AAACATTACG	CGAACACCCA	420
	GAACGTGCAC	AAGAAGTATT	CTCTGAATCA	CTTAAAGACA	GCAAGAACCC	AGACCGACGT	480
	GTTGCACAAC	AAAACGCTTT	TTACAATGTT	CTTAAAAATG	ATAACTTAAC	TGAACAAGAA	540
15	AAAAATAATT	ACATTGCACA	AATTAAAGAA	AACCCTGATA	GAAGCCAACA	AGTTTGGGTA	600
	GAATCAGTAC	AATCTTCTAA	AGCTAAAGAA	CGTCAAAATA	TTGAAAATGC	GGATAAAGCA	660
	ATTAAAGATT	TCCAAGATAA	CAAAGCACCA	CACGATAAAT	CAGCAGCATA	TGAAGCTAAC	720
	TCAAAATTAC	CTAAAGATTT	ACGTGATAAA	AACAACCGCT	TTGTAGAAAA	AGTTTCAATT	780
	GAAAAAGCAA	TCGTTTCGTCA	TGATGAGCGT	GTGAAATCAG	CAAATGATGC	AATCTCAAAA	840
20	TTAAATGAAA	AAGATTCAAT	TGAAAACAGA	CGTTTAGCAC	AACGTGAAGT	TAACAAAGCA	900
	CCTATGGATG	TAAAAGAGCA	TTTACAGAAA	CAATTAGACG	CATTAGTTGC	TCAAAAAGAT	960
	GCTGAAAAGA	AAGTGGCGCC	AAAAGTTGAG	GCTCCTCAAA	TTCAATCACC	ACAAATTGAA	1020
	AAACCTAAAG	TAGAATCACC	AAAAGTTGAA	GTCCCTCAAA	TTCAATCACC	AAAAGTTGAG	1080
	GTTCTCTCAAT	CTAAATTATT	AGGTTACTAC	CAATCATTAA	AAGATTCATT	TAACATGGT	1140
25	TACAAGTATT	TAACAGATAC	TTATAAAGC	TATAAAGAAA	AATATGATAC	AGCAAAGTAC	1200
	TACTATAATA	CGTACTATAA	ATACAAAGGT	GCGATTGATC	AAACAGTATT	AACAGTACTA	1260
	GGTAGTGGTT	CTAAATCTTA	CATCCAACCA	TTGAAAGTTG	ATGATAAAAA	CGGCTACTTA	1320
	GCTAAATCAT	ATGCACAAGT	AAGAACTAT	GTAAGTGAAGT	CAATCAATAC	TGGTAAAGTA	1380
	TTATATACTT	TCTACCAAAA	CCCAACATTA	GTAAAAACAG	CTATTAAAGC	TCAAGAACT	1440
30	GCATCATCAA	TCAAAAATAC	ATTAAGTAAT	TTATTATCAT	TCTGGAAATA	ATCAATCAAA	1500
	AATATCTTCT	CTAGTTTTAC	ATCATTTTTT	AAATAATTTT	CGTAACAAAC	CGTGATTAAA	1560
	AAGAACCGTT	GATTCTCAAT	CGAATCTACG	GTTCTTTTTT	CATTTTCCAT	CAATTAAATG	1620

- or a homologous sequence to SEQ ID NO:1 coding for said protein, or a partial or
35 homologous sequence of the sequence SEQ ID NO:1 coding for a polypeptide fragment of said protein comprising at least 15 amino acid residues.

2. Protein expressed by a bacterium of the genus *Staphylococcus aureus* or a polypeptide fragment of said protein comprising at least 15 amino acid residues other than the 84 aa fragment at the position 38 - 121, which protein comprises the amino acid sequence
40 SEQ ID NO: 2

	Met	Lys	Asn	Lys	Tyr	Ile	Ser	Lys	Leu	Leu	Val	Gly	Ala	Ala	Thr	Ile	
	1				5				10					15			
	Thr	Leu	Ala	Thr	Met	Ile	Ser	Asn	Gly	Glu	Ala	Lys	Ala	Ser	Glu	Asn	
				20					25				30				
45	Thr	Gln	Gln	Thr	Ser	Thr	Lys	His	Gln	Thr	Thr	Gln	Asn	Asn	Tyr	Val	
			35				40					45					
	Thr	Asp	Gln	Gln	Lys	Ala	Phe	Tyr	Gln	Val	Leu	His	Leu	Lys	Gly	Ile	
		50				55				60							
50	Thr	Glu	Glu	Gln	Arg	Asn	Gln	Tyr	Ile	Lys	Thr	Leu	Arg	Glu	His	Pro	
	65				70				75						80		

	Glu	Arg	Ala	Gln	Glu	Val	Phe	Ser	Glu	Ser	Leu	Lys	Asp	Ser	Lys	Asn	
				85					90						95		
	Pro	Asp	Arg	Arg	Val	Ala	Gln	Gln	Asn	Ala	Phe	Tyr	Asn	Val	Leu	Lys	
			100					105					110				
5	Asn	Asp	Asn	Leu	Thr	Glu	Gln	Glu	Lys	Asn	Asn	Tyr	Ile	Ala	Gln	Ile	
		115						120					125				
	Lys	Glu	Asn	Pro	Asp	Arg	Ser	Gln	Gln	Val	Trp	Val	Glu	Ser	Val	Gln	
		130					135					140					
10	Ser	Ser	Lys	Ala	Lys	Glu	Arg	Gln	Asn	Ile	Glu	Asn	Ala	Asp	Lys	Ala	
	145				150						155					160	
	Ile	Lys	Asp	Phe	Gln	Asp	Asn	Lys	Ala	Pro	His	Asp	Lys	Ser	Ala	Ala	
				165						170						175	
	Tyr	Glu	Ala	Asn	Ser	Lys	Leu	Pro	Lys	Asp	Leu	Arg	Asp	Lys	Asn	Asn	
				180					185					190			
15	Arg	Phe	Val	Glu	Lys	Val	Ser	Ile	Glu	Lys	Ala	Ile	Val	Arg	His	Asp	
		195						200					205				
	Glu	Arg	Val	Lys	Ser	Ala	Asn	Asp	Ala	Ile	Ser	Lys	Leu	Asn	Glu	Lys	
		210					215					220					
20	Asp	Ser	Ile	Glu	Asn	Arg	Arg	Leu	Ala	Gln	Arg	Glu	Val	Asn	Lys	Ala	
	225				230						235					240	
	Pro	Met	Asp	Val	Lys	Glu	His	Leu	Gln	Lys	Gln	Leu	Asp	Ala	Leu	Val	
				245						250						255	
	Ala	Gln	Lys	Asp	Ala	Glu	Lys	Lys	Val	Ala	Pro	Lys	Val	Glu	Ala	Pro	
				260					265					270			
25	Gln	Ile	Gln	Ser	Pro	Gln	Ile	Glu	Lys	Pro	Lys	Val	Glu	Ser	Pro	Lys	
			275					280						285			
	Val	Glu	Val	Pro	Gln	Ile	Gln	Ser	Pro	Lys	Val	Glu	Val	Pro	Gln	Ser	
		290					295					300					
30	Lys	Leu	Leu	Gly	Tyr	Tyr	Gln	Ser	Leu	Lys	Asp	Ser	Phe	Asn	Tyr	Gly	
	305				310						315					320	
	Tyr	Lys	Tyr	Leu	Thr	Asp	Thr	Tyr	Lys	Ser	Tyr	Lys	Glu	Lys	Tyr	Asp	
				325						330						335	
	Thr	Ala	Lys	Tyr	Tyr	Asn	Thr	Tyr	Tyr	Lys	Tyr	Lys	Gly	Ala	Ile		
				340				345						350			
35	Asp	Gln	Thr	Val	Leu	Thr	Val	Leu	Gly	Ser	Gly	Ser	Lys	Ser	Tyr	Ile	
			355					360					365				
	Gln	Pro	Leu	Lys	Val	Asp	Asp	Lys	Asn	Gly	Tyr	Leu	Ala	Lys	Ser	Tyr	
		370				375						380					
40	Ala	Gln	Val	Arg	Asn	Tyr	Val	Thr	Glu	Ser	Ile	Asn	Thr	Gly	Lys	Val	
	385				390						395					400	
	Leu	Tyr	Thr	Phe	Tyr	Gln	Asn	Pro	Thr	Leu	Val	Lys	Thr	Ala	Ile	Lys	
				405						410					415		
	Ala	Gln	Glu	Thr	Ala	Ser	Ser	Ile	Lys	Asn	Thr	Leu	Ser	Asn	Leu	Leu	
				420					425					430			
45	Ser	Phe	Trp	Lys.													
			435														

or a homologous sequence to the sequence SEQ ID NO: 2 comprising a few mismatches in the amino acid sequence of SEQ ID NO: 2, or polypeptide fragments of said homologous sequence comprising at least 15 amino acid residues.

3. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 3

	Thr	Gln	Gln	Thr	Ser	Thr	Lys	His	Gln	Thr	Thr	Gln	Asn	Asn	Tyr	Val
	1				5					10					15	
5	Thr	Asp	Gln	Gln	Lys	Ala	Phe	Tyr	Gln	Val	Leu	His	Leu	Lys	Gly	Ile
			20						25					30		
	Thr	Glu	Glu	Gln	Arg	Asn	Gln	Tyr	Ile	Lys	Thr	Leu	Arg	Glu	His	Pro
		35						40					45			
	Glu	Arg	Ala	Gln	Glu	Val	Phe	Ser	Glu	Ser	Leu	Lys	Asp	Ser	Lys	Asn
	50						55					60				
10	Pro	Asp	Arg	Arg	Val	Ala	Gln	Gln	Asn	Ala	Phe	Tyr	Asn	Val	Leu	Lys
	65				70						75					80
	Asn	Asp	Asn	Leu	Thr	Glu	Gln	Glu	Lys	Asn	Asn	Tyr	Ile	Ala	Gln	Ile
				85					90						95	
15	Lys	Glu	Asn	Pro	Asp	Arg	Ser	Gln	Gln	Val	Trp	Val	Glu	Ser	Val	Gln
			100						105					110		
	Ser	Ser	Lys	Ala	Lys	Glu	Arg	Gln	Asn	Ile	Glu	Asn	Ala	Asp	Lys	Ala
		115						120					125			
	Ile	Lys	Asp	Phe	Gln	Asp	Asn	Lys	Ala	Pro	His	Asp	Lys	Ser	Ala	Ala
	130						135					140				
20	Tyr	Glu	Ala	Asn	Ser	Lys	Leu	Pro	Lys	Asp	Leu	Arg	Asp	Lys	Asn	Asn
	145				150						155					160
	Arg	Phe	Val	Glu	Lys	Val	Ser	Ile	Glu	Lys	Ala	Ile	Val	Arg	His	Asp
				165					170						175	
25	Glu	Arg	Val	Lys	Ser	Ala	Asn	Asp	Ala	Ile	Ser	Lys	Leu	Asn	Glu	Lys
			180						185					190		
	Asp	Ser	Ile	Glu	Asn	Arg	Arg	Leu	Ala	Gln	Arg	Glu	Val	Asn	Lys	Ala
		195						200					205			
	Pro	Met	Asp	Val	Lys	Glu	His	Leu	Gln	Lys	Gln	Leu	Asp	Ala	Leu	Val
	210						215					220				
30	Ala	Gln	Lys	Asp	Ala	Glu	Lys	Lys	Val	Ala	Pro	Lys	Val	Glu	Ala	Pro
	225				230						235					240
	Gln	Ile	Gln	Ser	Pro	Gln	Ile	Glu	Lys	Pro	Lys	Val	Glu	Ser	Pro	Lys
				245					250						255	
35	Val	Glu	Val	Pro	Gln	Ile	Gln	Ser	Pro	Lys	Val	Glu	Val	Pro	Gln	Ser
			260						265					270		
	Lys	Leu	Leu	Gly	Tyr	Tyr	Gln	Ser	Leu	Lys	Asp	Ser	Phe	Asn	Tyr	Gly
		275					280						285			
	Tyr	Lys	Tyr	Leu	Thr	Asp	Thr	Tyr	Lys	Ser	Tyr	Lys	Glu	Lys	Tyr	Asp
	290					295						300				
40	Thr	Ala	Lys	Tyr	Tyr	Tyr	Asn	Thr	Tyr	Tyr	Lys	Tyr	Lys	Gly	Ala	Ile
	305					310					315					320
	Asp	Gln	Thr	Val	Leu	Thr	Val	Leu	Gly	Ser	Gly	Ser	Lys	Ser	Tyr	Ile
				325					330						335	
45	Gln	Pro	Leu	Lys	Val	Asp	Asp	Lys	Asn	Gly	Tyr	Leu	Ala	Lys	Ser	Tyr
			340						345					350		
	Ala	Gln	Val	Arg	Asn	Tyr	Val	Thr	Glu	Ser	Ile	Asn	Thr	Gly	Lys	Val
		355						360					365			
	Leu	Tyr	Thr	Phe	Tyr	Gln	Asn	Pro	Thr	Leu	Val	Lys	Thr	Ala	Ile	Lys
	370					375						380				
50	Ala	Gln	Glu	Thr	Ala	Ser	Ser	Ile	Lys	Asn	Thr	Leu	Ser	Asn	Leu	Leu
	385					390					395					400
	Ser	Phe	Trp	Lys.												

4. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 4

5	Val	Gln	Ser	Ser	Lys	Ala	Lys	Glu	Arg	Gln	Asn	Ile	Glu	Asn	Ala	Asp
	1				5					10					15	
	Lys	Ala	Ile	Lys	Asp	Phe	Gln	Asp	Asn	Lys	Ala	Pro	His	Asp	Lys	Ser
				20					25					30		
	Ala	Ala	Tyr	Glu	Ala	Asn	Ser	Lys	Leu	Pro	Lys	Asp	Leu	Arg	Asp	Lys
				35					40					45		
10	Asn	Asn	Arg	Phe	Val	Glu	Lys	Val	Ser	Ile	Glu	Lys	Ala	Ile	Val	Arg
		50					55						60			
	His	Asp	Glu	Arg	Val	Lys	Ser	Ala	Asn	Asp	Ala	Ile	Ser	Lys	Leu	Asn
	65					70					75				80	
	Glu	Lys	Asp	Ser	Ile	Glu	Asn	Arg	Arg	Leu	Ala	Gln	Arg	Glu	Val	Asn
15					85					90					95	
	Lys	Ala	Pro	Met	Asp	Val	Lys	Glu	His	Leu	Gln	Lys	Gln	Leu	Asp	Ala
				100					105					110		
	Leu	Val	Ala	Gln	Lys	Asp	Ala	Glu	Lys	Lys	Val	Ala	Pro	Lys	Val	Glu
				115					120					125		
20	Ala	Pro	Gln	Ile	Gln	Ser	Pro	Gln	Ile	Glu	Lys	Pro	Lys	Val	Glu	Ser
		130					135						140			
	Pro	Lys	Val	Glu	Val	Pro	Gln	Ile	Gln	Ser	Pro	Lys	Val	Glu	Val	Pro
	145					150						155				160
	Gln	Ser	Lys	Leu	Leu	Gly	Tyr	Tyr	Gln	Ser	Leu	Lys	Asp	Ser	Phe	Asn
25					165					170					175	
	Tyr	Gly	Tyr	Lys	Tyr	Leu	Thr	Asp	Thr	Tyr	Lys	Ser	Tyr	Lys	Glu	Lys
				180					185						190	
	Tyr	Asp	Thr	Ala	Lys	Tyr	Tyr	Tyr	Asn	Thr	Tyr	Tyr	Lys	Tyr	Lys	Gly
				195					200					205		
30	Ala	Ile	Asp	Gln	Thr	Val	Leu	Thr	Val	Leu	Gly	Ser	Gly	Ser	Lys	Ser
		210					215						220			
	Tyr	Ile	Gln	Pro	Leu	Lys	Val	Asp	Asp	Lys	Asn	Gly	Tyr	Leu	Ala	Lys
	225					230					235					240
	Ser	Tyr	Ala	Gln	Val	Arg	Asn	Tyr	Val	Thr	Glu	Ser	Ile	Asn	Thr	Gly
35					245					250					255	
	Lys	Val	Leu	Tyr	Thr	Phe	Tyr	Gln	Asn	Pro	Thr	Leu	Val	Lys	Thr	Ala
				260					265					270		
	Ile	Lys	Ala	Gln	Glu	Thr	Ala	Ser	Ser	Ile	Lys	Asn	Thr	Leu	Ser	Asn
				275				280						285		
40	Leu	Leu	Ser	Phe	Trp	Lys.										
		290														

5. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 5

45	Glu	Asn	Thr	Gln	Gln	Thr	Ser	Thr	Lys	His	Gln	Thr	Thr	Gln	Asn	Asn
	1				5					10					15	
	Tyr	Val	Thr	Asp	Gln	Gln	Lys	Ala	Phe	Tyr	Gln	Val	Leu	His	Leu	Lys
				20					25					30		
	Gly	Ile	Thr	Glu	Glu	Gln	Arg	Asn	Gln	Tyr	Ile	Lys	Thr	Leu	Arg	Glu
50				35				40					45			
	His	Pro	Glu	Arg	Ala	Gln	Glu	Val	Phe	Ser	Glu	Ser	Leu	Lys	Asp	Ser
		50					55					60				

Lys Asn Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val
 65 70 75 80
 Leu Lys Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala
 5 85 90 95
 Gln Ile Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser
 100 105 110
 Val Gln Ser Ser Lys Ala Lys Glu.
 115 120

6. Polypeptide fragment of the protein according to claim 2 having the amino acid sequence SEQ ID NO: 6

Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala
 1 5 10 15
 Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala
 15 20 25 30
 Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn
 35 40 45
 Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp
 20 50 55 60
 Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys
 65 70 75 80
 Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala
 85 90 95
 Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val
 25 100 105 110
 Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro.
 115 120

7. Protein or polypeptide according to any one of claims 2 – 6 coupled to an inert carrier or matrix.

8. Vector selected from the group consisting of plasmids, phages or phagemides comprising a DNA molecule according to claim 1 or the corresponding RNA molecule.

9. Antibodies specifically binding to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4, and SEQ ID NO:6.

10. Use of a protein or polypeptide according to any one of claims 2 – 7 as an immunising component in the production of a vaccine against Staphylococcal infections.

11. Use of a vector according to claim 8 for the production of a vaccine against Staphylococcal infections.

12. Use of antibodies according to claim 9 for the production of a vaccine for the passive immunisation of a mammal against Staphylococcal infections.

13. Vaccine against Staphylococcal infections comprising as an immunising component a protein or polypeptide according to any one of claims 2 - 7.

14. Vaccine against Staphylococcal infections comprising a vector according to claim 8.

15. Vaccine for the passive immunisation of a mammal against Staphylococcal infections comprising antibodies according to claim 9.

5 16. Method of prophylactic and/or therapeutic treatment of Staphylococcal infections in a mammal comprising administration to said mammal of an immunologically effective amount of a vaccine according to any one of claims 12 –14.

10 17. Method of isolating and/or purifying apolipoprotein H from a liquid medium, especially from serum, comprising chromatographic separation of apolipoprotein H from said liquid medium with an immobilised protein or polypeptide according to claim 7 as the stationary phase.

1/4

a.



b.

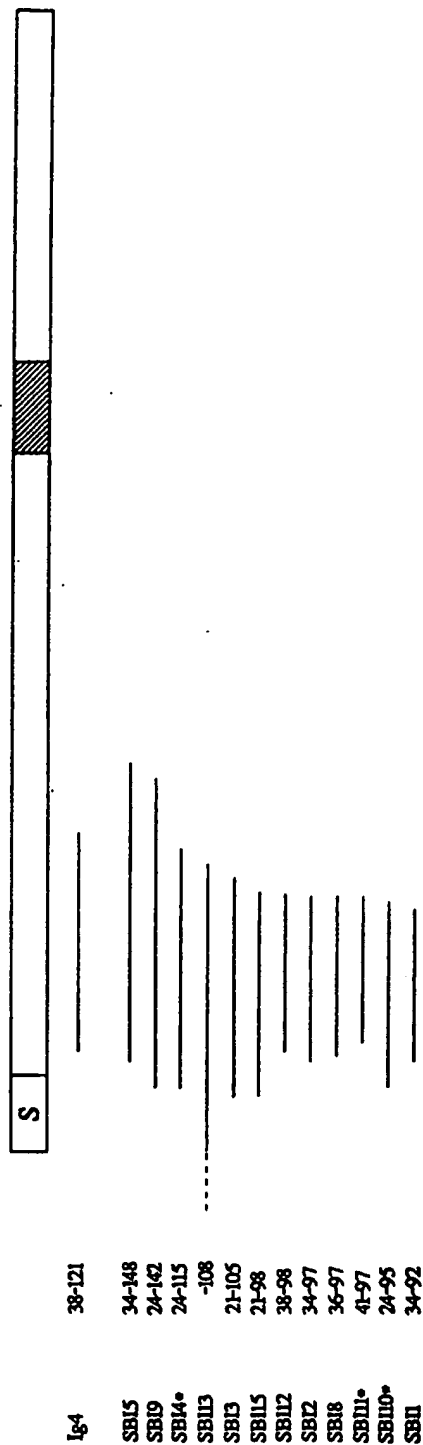


Fig. 1

AGTACTTCCTTACTTAAAATACGCTGAATGTTCTGAATTAAACGCTTTTTTACATAGTTA 60
ACACTAGTTAATCTATTAGTTAACATTAGTTAATAATTAGTTAATTTCCATTTGTATTCT 120
CATGTGATAAATTCTAAAAGCATAACAATAAATTTAATATGTAAAAAGAAAGGGAATACAC 180
ATGAAAAATAAATATATCTCGAAGTTGCTAGTTGGGGCAGCAACAATTACGTTAGCTACA 240
1 M K N K Y I S K L L V G A A T I T L A T
ATGATTTCAAATGGGGAAGCAAAAGCGAGTGAAAACACGCAACAACTTCAACTAAGCAC 300
21 M I S N G E A K A S E N T Q Q T S T K H
CAAACAACCTCAAAACAACCTACGTAACAGATCAACAAAAAGCTTTTTATCAAGTATTACAT 360
41 Q T T Q N N Y V T D Q Q K A F Y Q V L H
CTAAAAGGTATCACAGAAGAACAACGTAACCAATACATCAAAACATTACGCGAACACCCA 420
61 L K G I T E E Q R N Q Y I K T L R E H P
GAACGTGCACAAGAAGTATTCTCTGAATCACTTAAAGACAGCAAGAACCCAGACCGACGT 480
81 E R A Q E V F S E S L K D S K N P D R R
GTTGCACAACAAAACGCTTTTTTACAATGTTCTTAAAAATGATAACTTAACTGAACAAGAA 540
101 V A Q Q N A F Y N V L K N D N L T E Q E
AAAAATAATTACATTGCACAAATTAAAGAAAACCCCTGATAGAAGCCAACAAGTTTGGGTA 600
121 K N N Y I A Q I K E N P D R S Q Q V W V
GAATCAGTACAATCTTCTAAAGCTAAAGAACGTCAAAATATTGAAAATGCGGATAAAGCA 660
141 E S V Q S S K A K E R Q N I E N A D K A
ATTAAAGATTTCCAAGATAACAAAGCACCACACGATAAATCAGCAGCATATGAAGCTAAC 720
161 I K D F Q D N K A P H D K S A A Y E A N
TCAAAATTACCTAAAGATTTACGTGATAAAAACAACCGCTTTGTAGAAAAAGTTTCAATT 780
181 S K L P K D L R D K N N R F V E K V S I
GAAAAAGCAATCGTTCGTATGATGAGCGTGTGAAATCAGCAAATGATGCAATCTCAAAA 840
201 E K A I V R H D E R V K S A N D A I S K
TTAAATGAAAAAGATTCAATTGAAAACAGACGTTTAGCACAACGTGAAGTTAACAAAGCA 900
221 L N E K D S I E N R R L A Q R E V N K A

Fig. 2a

CCTATGGATGTAAAAGAGCATTACAGAAACAATTAGACGCATTAGTTGCTCAAAAAGAT 960
241 P M D V K E H L Q K Q L D A L V A Q K D

GCTGAAAAGAAAGTGGCGCCAAAAGTTGAGGCTCCTCAAATTCAATCACCACAAATTGAA 1020
261 A E K K V A P K V E A P Q I Q S P Q I E

AAACCTAAAGTAGAATCACCAAAAGTTGAAGTCCCTCAAATTCAATCACCAAAAGTTGAG 1080
281 K P K V E S P K V E V P Q I Q S P K V E

GTTCTCAATCTAAATTATTAGGTTACTACCAATCATTAAAAGATTCAATTAACTATGGT 1140
301 V P Q S K L L G Y Y Q S L K D S F N Y G

TACAAGTATTTAACAGATACTTATAAAAGCTATAAAGAAAAATATGATACAGCAAAGTAC 1200
321 Y K Y L T D T Y K S Y K E K Y D T A K Y

TACTATAATACGTACTATAAATACAAAGGTGCGATTGATCAAACAGTATTAACAGTACTA 1260
341 Y Y N T Y Y K Y K G A I D Q T V L T V L

GGTAGTGGTTCTAAATCTTACATCCAACCATTGAAAGTTGATGATAAAAACGGCTACTTA 1320
361 G S G S K S Y I Q P L K V D D K N G Y L

GCTAAATCATATGCACAAGTAAGAACTATGTAAGTCAATCAATACTGGTAAAGTA 1380
381 A K S Y A Q V R N Y V T E S I N T G K V

TTATATACTTTCTACCAAAACCCAACATTAGTAAAAACAGCTATTAAAGCTCAAGAACT 1440
401 L Y T F Y Q N P T L V K T A I K A Q E T

GCATCATCAATCAAAAATACATTAAGTAATTTATTATCATTCTGGAAATAATCAATCAAA 1500
421 A S S I K N T L S N L L S F W K

AATATCTTCTCTAGTTTTACATCATTTTTTAAATAATTTTCGTAACAAACCGTGATTAAA 1560
AAGAACCGTTGATTCTCAATCGAATCTACGGTTCTTTTTTCATTTTCCATCAATTAAATG 1620

Fig. 2b

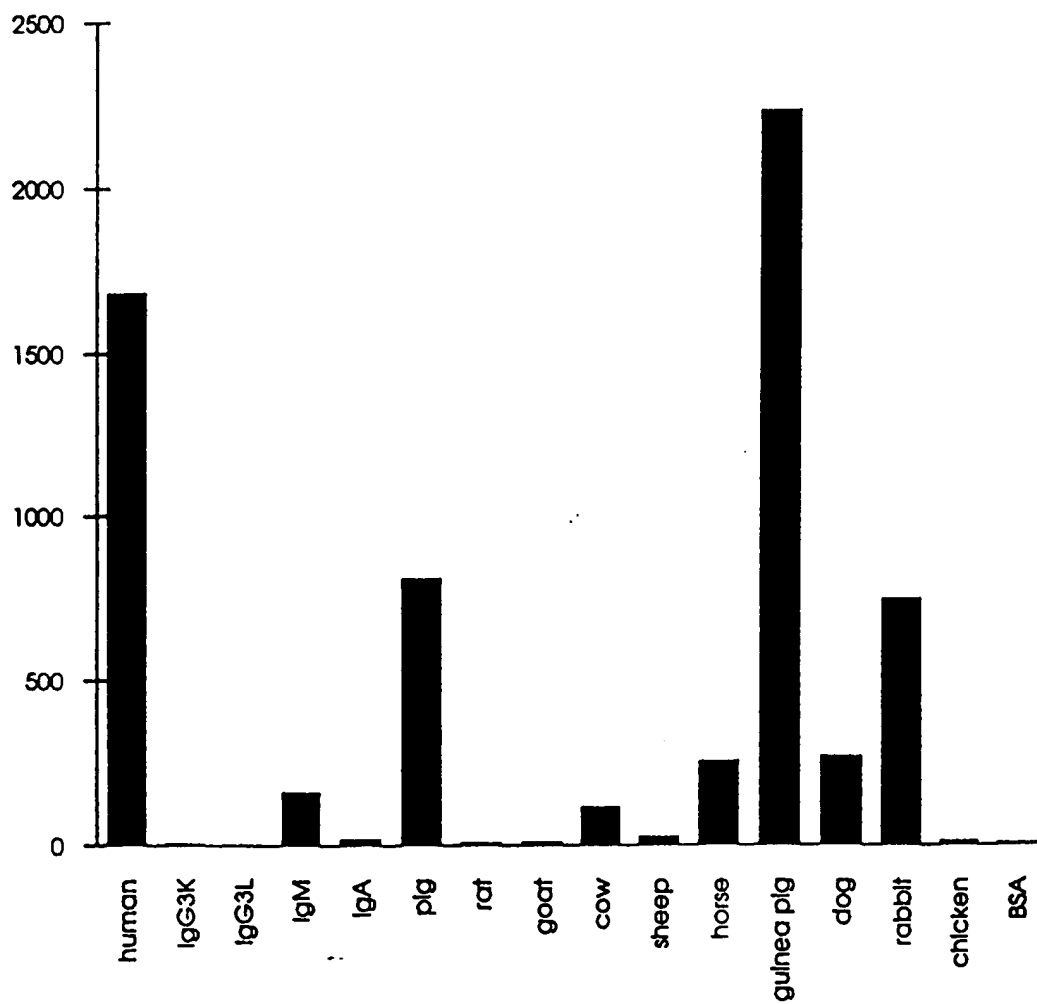


Fig. 3

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: SBL Vaccin AB (Publ.)
(B) STREET: Lundagatan 2
(C) CITY: Stockholm
(E) COUNTRY: Sweden
(F) POSTAL CODE (ZIP): 105 21

(ii) TITLE OF INVENTION: New protein and nucleotide sequence encoding
said protein

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: SE 9704141-2
(B) FILING DATE: 12-NOV-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1620 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGTACTTCCT TACTTAAAT ACGCTGAATG TTCTGAATTA AACGCTTTT TACATAGTTA	60
ACACTAGTTA ATCTATTAGT TAACATTAGT TAATAATTAG TTAATTTCCA TTTGTATTCT	120
CATGTGATAA ATTCTAAAAG CATAACAATA ATTTAATATG TAAAAAGAAA GGAATACAC	180
ATGAAAAATA AATATATCTC GAAGTTGCTA GTTGGGGCAG CAACAATTAC GTTAGCTACA	240
ATGATTTCAA ATGGGGAAGC AAAAGCGAGT GAAAACACGC AACAACTTC AACTAAGCAC	300
CAAACAATC AAAACAATA CGTAACAGAT CAACAAAAG CTTTTTATCA AGTATTACAT	360
CTAAAAGGTA TCACAGAAGA ACAACGTAAC CAATACATCA AAACATTACG CGAACACCCA	420
GAACGTGCAC AAGAAGTATT CTCTGAATCA CTTAAAGACA GCAAGAACCC AGACCGACGT	480

5 GTTGACACAAC AAAACGCTTT TTACAATGTT CTTAAAAATG ATAACTTAAC TGAACAAGAA 540
AAAAATAATT ACATTGCACA AATTAAAGAA AACCTGATA GAAGCCAACA AGTTTGGGTA 600
GAATCAGTAC AATCTTCTAA AGCTAAAGAA CGTCAAAATA TTGAAAATGC GGATAAAGCA 660
ATTAAAGATT TCCAAGATAA CAAAGCACCA CACGATAAAT CAGCAGCATA TGAAGCTAAC 720
10 TCAAAATTAC CTAAAGATTT ACGTGATAAA AACAACCGCT TTGTAGAAAA AGTTTCAATT 780
GAAAAAGCAA TCGTTCGTCA TGATGAGCGT GTGAAATCAG CAAATGATGC AATCTCAAAA 840
TTAAATGAAA AAGATTCAAT TGAAAACAGA CGTTTAGCAC AACGTGAAGT TAACAAAGCA 900
15 CCTATGGATG TAAAAGAGCA TTTACAGAAA CAATTAGACG CATTAGTTGC TCAAAAAGAT 960
GCTGAAAAGA AAGTGGCGCC AAAAGTTGAG GCTCCTCAAA TTCAATCACC ACAAATTGAA 1020
20 AAACCTAAAG TAGAATCACC AAAAGTTGAA GTCCCTCAAA TTCAATCACC AAAAGTTGAG 1080
GTTCTCAAT CTAAATTATT AGGTTACTAC CAATCATTAA AAGATTCATT TAACTATGGT 1140
TACAAGTATT TAACAGATAC TTATAAAGC TATAAAGAAA AATATGATAC AGCAAAGTAC 1200
25 TACTATAATA CGTACTATAA ATACAAAGGT GCGATTGATC AAACAGTATT AACAGTACTA 1260
GGTAGTGGTT CTAAATCTTA CATCCAACCA TTGAAAGTTG ATGATAAAAA CGGCTACTTA 1320
30 GCTAAATCAT ATGCACAAGT AAGAACTAT GTAAGTGAAGT CAATCAATAC TGGTAAAGTA 1380
TTATATACTT TCTACCAAAA CCCAACATTA GTAAAAACAG CTATTAAAGC TCAAGAACT 1440
GCATCATCAA TCAAAAATAC ATTAAGTAAT TTATTATCAT TCTGGAAATA ATCAATCAAA 1500
35 AATATCTTCT CTAGTTTTAC ATCATTTTTT AAATAATTTT CGTAACAAAC CGTGATTAAA 1560
AAGAACCGTT GATTCTCAAT CGAATCTACG GTTCTTTTTT CATTTTCCAT CAATTAAATG 1620

40

(2) INFORMATION FOR SEQ ID NO: 2:

45

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

50

- (ii) MOLECULE TYPE: protein
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

5	Met Lys Asn Lys Tyr Ile Ser Lys Leu Leu Val Gly Ala Ala Thr Ile	1 5 10 15
	Thr Leu Ala Thr Met Ile Ser Asn Gly Glu Ala Lys Ala Ser Glu Asn	20 25 30
10	Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val	35 40 45
	Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile	50 55 60
15	Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro	65 70 75 80
20	Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn	85 90 95
	Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys	100 105 110
25	Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala Gln Ile	115 120 125
	Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser Val Gln	130 135 140
30	Ser Ser Lys Ala Lys Glu Arg Gln Asn Ile Glu Asn Ala Asp Lys Ala	145 150 155 160
35	Ile Lys Asp Phe Gln Asp Asn Lys Ala Pro His Asp Lys Ser Ala Ala	165 170 175
	Tyr Glu Ala Asn Ser Lys Leu Pro Lys Asp Leu Arg Asp Lys Asn Asn	180 185 190
40	Arg Phe Val Glu Lys Val Ser Ile Glu Lys Ala Ile Val Arg His Asp	195 200 205
	Glu Arg Val Lys Ser Ala Asn Asp Ala Ile Ser Lys Leu Asn Glu Lys	210 215 220
45	Asp Ser Ile Glu Asn Arg Arg Leu Ala Gln Arg Glu Val Asn Lys Ala	225 230 235 240
50	Pro Met Asp Val Lys Glu His Leu Gln Lys Gln Leu Asp Ala Leu Val	245 250 255
	Ala Gln Lys Asp Ala Glu Lys Lys Val Ala Pro Lys Val Glu Ala Pro	260 265 270
55	Gln Ile Gln Ser Pro Gln Ile Glu Lys Pro Lys Val Glu Ser Pro Lys	275 280 285
60	Val Glu Val Pro Gln Ile Gln Ser Pro Lys Val Glu Val Pro Gln Ser	290 295 300

Lys Leu Leu Gly Tyr Tyr Gln Ser Leu Lys Asp Ser Phe Asn Tyr Gly
 305 310 315 320
 Tyr Lys Tyr Leu Thr Asp Thr Tyr Lys Ser Tyr Lys Glu Lys Tyr Asp
 5 325 330 335
 Thr Ala Lys Tyr Tyr Tyr Asn Thr Tyr Tyr Lys Tyr Lys Gly Ala Ile
 340 345 350
 Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser Tyr Ile
 10 355 360 365
 Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys Ser Tyr
 15 370 375 380
 Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly Lys Val
 385 390 395 400
 Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala Ile Lys
 20 405 410 415
 Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn Leu Leu
 420 425 430
 Ser Phe Trp Lys
 25 435

(2) INFORMATION FOR SEQ ID NO: 3:

- 30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 404 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 35 (D) TOPOLOGY: unknown
 (ii) MOLECULE TYPE: protein
 (v) FRAGMENT TYPE: C-terminal
 40 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Staphylococcus aureus*
 (C) INDIVIDUAL ISOLATE: 8325-4

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn Tyr Val
 1 5 10 15
 Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys Gly Ile
 50 20 25 30
 Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu His Pro
 55 35 40 45
 Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser Lys Asn
 50 55 60
 Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val Leu Lys
 60 65 70 75 80

	Asn	Asp	Asn	Leu	Thr	Glu	Gln	Glu	Lys	Asn	Asn	Tyr	Ile	Ala	Gln	Ile
					85					90					95	
5	Lys	Glu	Asn	Pro	Asp	Arg	Ser	Gln	Gln	Val	Trp	Val	Glu	Ser	Val	Gln
				100					105					110		
	Ser	Ser	Lys	Ala	Lys	Glu	Arg	Gln	Asn	Ile	Glu	Asn	Ala	Asp	Lys	Ala
			115					120					125			
10	Ile	Lys	Asp	Phe	Gln	Asp	Asn	Lys	Ala	Pro	His	Asp	Lys	Ser	Ala	Ala
		130					135					140				
	Tyr	Glu	Ala	Asn	Ser	Lys	Leu	Pro	Lys	Asp	Leu	Arg	Asp	Lys	Asn	Asn
15		145				150					155					160
	Arg	Phe	Val	Glu	Lys	Val	Ser	Ile	Glu	Lys	Ala	Ile	Val	Arg	His	Asp
					165					170					175	
20	Glu	Arg	Val	Lys	Ser	Ala	Asn	Asp	Ala	Ile	Ser	Lys	Leu	Asn	Glu	Lys
				180					185					190		
	Asp	Ser	Ile	Glu	Asn	Arg	Arg	Leu	Ala	Gln	Arg	Glu	Val	Asn	Lys	Ala
			195					200					205			
25	Pro	Met	Asp	Val	Lys	Glu	His	Leu	Gln	Lys	Gln	Leu	Asp	Ala	Leu	Val
		210					215					220				
	Ala	Gln	Lys	Asp	Ala	Glu	Lys	Lys	Val	Ala	Pro	Lys	Val	Glu	Ala	Pro
30		225				230					235					240
	Gln	Ile	Gln	Ser	Pro	Gln	Ile	Glu	Lys	Pro	Lys	Val	Glu	Ser	Pro	Lys
					245					250					255	
35	Val	Glu	Val	Pro	Gln	Ile	Gln	Ser	Pro	Lys	Val	Glu	Val	Pro	Gln	Ser
				260					265					270		
	Lys	Leu	Leu	Gly	Tyr	Tyr	Gln	Ser	Leu	Lys	Asp	Ser	Phe	Asn	Tyr	Gly
			275					280					285			
40	Tyr	Lys	Tyr	Leu	Thr	Asp	Thr	Tyr	Lys	Ser	Tyr	Lys	Glu	Lys	Tyr	Asp
		290					295						300			
	Thr	Ala	Lys	Tyr	Tyr	Tyr	Asn	Thr	Tyr	Tyr	Lys	Tyr	Lys	Gly	Ala	Ile
45		305				310					315					320
	Asp	Gln	Thr	Val	Leu	Thr	Val	Leu	Gly	Ser	Gly	Ser	Lys	Ser	Tyr	Ile
					325					330					335	
50	Gln	Pro	Leu	Lys	Val	Asp	Asp	Lys	Asn	Gly	Tyr	Leu	Ala	Lys	Ser	Tyr
				340					345					350		
	Ala	Gln	Val	Arg	Asn	Tyr	Val	Thr	Glu	Ser	Ile	Asn	Thr	Gly	Lys	Val
			355					360					365			
55	Leu	Tyr	Thr	Phe	Tyr	Gln	Asn	Pro	Thr	Leu	Val	Lys	Thr	Ala	Ile	Lys
		370					375					380				
	Ala	Gln	Glu	Thr	Ala	Ser	Ser	Ile	Lys	Asn	Thr	Leu	Ser	Asn	Leu	Leu
60		385				390					395					400

Ser Phe Trp Lys

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 294 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Staphylococcus aureus
(C) INDIVIDUAL ISOLATE: 8325-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Val	Gln	Ser	Ser	Lys	Ala	Lys	Glu	Arg	Gln	Asn	Ile	Glu	Asn	Ala	Asp	1	5	10	15
Lys	Ala	Ile	Lys	Asp	Phe	Gln	Asp	Asn	Lys	Ala	Pro	His	Asp	Lys	Ser	20	25	30	
Ala	Ala	Tyr	Glu	Ala	Asn	Ser	Lys	Leu	Pro	Lys	Asp	Leu	Arg	Asp	Lys	35	40	45	
Asn	Asn	Arg	Phe	Val	Glu	Lys	Val	Ser	Ile	Glu	Lys	Ala	Ile	Val	Arg	50	55	60	
His	Asp	Glu	Arg	Val	Lys	Ser	Ala	Asn	Asp	Ala	Ile	Ser	Lys	Leu	Asn	65	70	75	80
Glu	Lys	Asp	Ser	Ile	Glu	Asn	Arg	Arg	Leu	Ala	Gln	Arg	Glu	Val	Asn	85	90	95	
Lys	Ala	Pro	Met	Asp	Val	Lys	Glu	His	Leu	Gln	Lys	Gln	Leu	Asp	Ala	100	105	110	
Leu	Val	Ala	Gln	Lys	Asp	Ala	Glu	Lys	Lys	Val	Ala	Pro	Lys	Val	Glu	115	120	125	
Ala	Pro	Gln	Ile	Gln	Ser	Pro	Gln	Ile	Glu	Lys	Pro	Lys	Val	Glu	Ser	130	135	140	
Pro	Lys	Val	Glu	Val	Pro	Gln	Ile	Gln	Ser	Pro	Lys	Val	Glu	Val	Pro	145	150	155	160
Gln	Ser	Lys	Leu	Leu	Gly	Tyr	Tyr	Gln	Ser	Leu	Lys	Asp	Ser	Phe	Asn	165	170	175	
Tyr	Gly	Tyr	Lys	Tyr	Leu	Thr	Asp	Thr	Tyr	Lys	Ser	Tyr	Lys	Glu	Lys	180	185	190	
Tyr	Asp	Thr	Ala	Lys	Tyr	Tyr	Tyr	Asn	Thr	Tyr	Tyr	Lys	Tyr	Lys	Gly				

195 200 205

Ala Ile Asp Gln Thr Val Leu Thr Val Leu Gly Ser Gly Ser Lys Ser
210 215 220

5 Tyr Ile Gln Pro Leu Lys Val Asp Asp Lys Asn Gly Tyr Leu Ala Lys
225 230 235 240

10 Ser Tyr Ala Gln Val Arg Asn Tyr Val Thr Glu Ser Ile Asn Thr Gly
245 250 255

Lys Val Leu Tyr Thr Phe Tyr Gln Asn Pro Thr Leu Val Lys Thr Ala
260 265 270

15 Ile Lys Ala Gln Glu Thr Ala Ser Ser Ile Lys Asn Thr Leu Ser Asn
275 280 285

Leu Leu Ser Phe Trp Lys
290

20

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 120 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (v) FRAGMENT TYPE: internal
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Staphylococcus aureus
 (C) INDIVIDUAL ISOLATE: 8325-4
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

40 Glu Asn Thr Gln Gln Thr Ser Thr Lys His Gln Thr Thr Gln Asn Asn
1 5 10 15

Tyr Val Thr Asp Gln Gln Lys Ala Phe Tyr Gln Val Leu His Leu Lys
20 25 30

45 Gly Ile Thr Glu Glu Gln Arg Asn Gln Tyr Ile Lys Thr Leu Arg Glu
35 40 45

His Pro Glu Arg Ala Gln Glu Val Phe Ser Glu Ser Leu Lys Asp Ser
50 55 60

Lys Asn Pro Asp Arg Arg Val Ala Gln Gln Asn Ala Phe Tyr Asn Val
65 70 75 80

55 Leu Lys Asn Asp Asn Leu Thr Glu Gln Glu Lys Asn Asn Tyr Ile Ala
85 90 95

Gln Ile Lys Glu Asn Pro Asp Arg Ser Gln Gln Val Trp Val Glu Ser
100 105 110

60 Val Gln Ser Ser Lys Ala Lys Glu
115 120

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/02036

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/31, A61K 39/085

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched*

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Biotechniques, Volume 18, No 5, 1995, Karin Jacobsson et al, "Cloning of Ligand-Binding Domains of Bacterial Receptors by Phage Display", page 3 - page 9, figure 2, and the whole document --	1-3,5,7-8, 10-14
X	Databas Swissprot:Spa2_Staau, accession no. P38507, Shuttleworth H.L. et al: "Immunoglobulin G binding protein A precursor (protein A)"; & Gene 58:283-295 (1987) --	1
P,X	Microbiology, Volume 144, 1998, Lihong Zhang et al, "A second IgG-binding protein in Staphylococcus aureus" page 985 - page 991 -- -----	1-15,17

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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Date of the actual completion of the international search

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Name and mailing address of the ISA/

Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

Patrick Andersson

Telephone No. +46 8 782 25 00